IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re- application of Jacques Mallet

Serial No. 10/511,343

Group art Unit: 1633
Examiner: Fereydoun G Sajjadi

Filed 04/11/2005

For: "*Optimization of transgene expression in mammalian cells.*"

DECLARATION UNDER RULE 132

Hon. Commissioner of Patents and Trademarks
WASHINGTON D.C. 20231

Sir:

I, Jacques Mallet, residing at 18, rue Charcot, 75013 PARIS, France.

Declare and Say:

I am a citizen of France.

I am Director of research in the CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE (CNRS), head of the LGN laboratory (Laboratoire de génétique moléculaire de la neurotransmission et des processus neurodégénératifs).

I am an inventor of the above identified patent application (Serial No. 10/511,343).

I am also named as author in the article entitled "*Optimization of transgene expression at the posttranscriptional level in neural cells: implications for gene therapy*" (**Brun et al.**; Molecular therapy, Vol. 7, No. 6, June 2003);

- I read the Office Action issued on June 16, 2009 by the USPTO as well as the cited prior art documents Barry et al., Paulding et al., Aronov et al., Rogers et al., Ramezani et al., and Chang et al.

In the present invention our aim was to provide a vector allowing high and long expression of its transgene, in order to improve the expression per molecule of vector and thereby reduce the toxicity of such a vector.

Using posttranscriptionnal regulatory elements described in the art, particular combinations of selected elements have been tested in plasmid or viral vectors in order to determine their respective effects on transgene expression in neuronal and glial cells. Some of them exhibit an increased effect, others an additive or a synergistic effect, depending both on the nature of the posttranscriptionnal regulatory elements and on the nature of the cell. Particularly advantageous combinations have been identified among the tested combinations.

As confirmed by the previously submitted publication of Brun et al., our aim was advantageously reached, in particular in **neuronal cells**, using a vector comprising in particular a chimeric genetic construct comprising a transgene operably linked to at least two distinct posttranscriptional regulatory elements functional in cells, wherein one of said posttranscriptional regulatory elements is **the tau 3'UTR** region of a eukaryotic mRNA or a functional portion thereof, and the other one is all or a functional portion of a **WPRE element**.

Figure 7 of our patent application, which is identical to Table 1 of Brun et al. (page 785), show the effects of WPRE, tau 3'UTR, TH 3'UTR and APP 5'UTR on luciferase expression.
Figure 7 is to be interpreted, as explained on page 18 of the patent application (lines 1-11), in the following way: "Values of relative luciferase activities obtained for each construct containing tau 3'UTR, TH 3'UTR, APP 5'UTR and WPRE, alone or in combination, are presented (data are graphically represented in the degree to which the effects of each element is cumulative on expression when they were combined. This rate was calculated by dividing the relative luciferase activity obtained with the combination of elements by the product of the effect of each element. It is expressed as a percentage. A rate of 100% indicates that the elements have the same effects on enhancement of expression when they were combined as when they were alone in the UTR of the luciferase transcript. *nd*, not determined."

As indicated in the patent application (see page 20, lines 27-28), "All the constructions were made in the pGL3-Control vector, which was used as a control plasmid.".
Brun et al. also explains that "*Luciferase expression from the pGL3-Control vector was arbitrarily defined as the value 1.0 and the activity of all vectors is expressed relative to this value*" (see right column, page 783).

The following examples of luciferase expressions are provided by Figure 7:

- the use of **Tau** alone leads to a luciferase expression of 1.53 in PC12 cells and 1.77 in NGF-PC12 cells,
- the use of **WPRE** alone leads to a luciferase expression of 6.86 in PC12 cells and 4.13 in NGF-PC12 cells,
- the use of **WPRE-Tau** leads to a luciferase expression of 16.8 in PC12 cells and 5.27 in NGF-PC12 cells,
- the use of **WPRE-APP-Tau** leads to a luciferase expression of 21.0 in PC12 cells and 15.5 in NGF-PC12 cells, and
- the use of **WPRE-APP-Tau-TH** leads to a luciferase expression of 26.0 in PC12 cells and 25.4 in NGF-PC12 cells.

In other words, in PC12 cells (also true when considering the increase of luciferase expression in NGF-PC12 cells), a higher increase of luciferase expression than the increase of expression obtained using Tau alone or WPRE alone (when compared to the expression from the pGL3-Control vector), is obtained when Tau is combined to WPRE. An even higher increase of expression is obtained when APP is combined to Tau and WPRE, the highest increase of expression being obtained when Tau, WPRE, APP and TH are used together.
An **additive effect** (which would already be very appreciable) of Tau and WPRE would lead to an increase of the luciferase expression of about 1.53×6.86 = 10.49, in PC12 cells for example.
The increase of luciferase expression actually observed (see Figure 7, line 8) is however of 16.8. Such an increase is the result of a **synergistic effect** of Tau and WPRE.

It is to note that combinations exhibiting **increased** or **additive effects** are also **of great interest** as they can be used in a vector according to the present invention for transgene delivery into particular mammalian cells.
Vectors comprising WPRE and both tau 3'UTR and APP 5'UTR in particular greatly enhance the expression of the transgene in mammalian **neuronal** cells. Brun et al. confirm that they have an additive effect on the transgene expression: "*In neuronal cells, WPRE and both tau 3'UTR and APP 5'UTR had an additive effect on expression*" (see abstract).
The additive effect of WPRE and both tau 3'UTR and APP 5'UTR should be respectively of about 19.83 (1.53×1.89×6.86) in PC12 cells, of about 16.00 in-PC12 cells and of about 26.53 in SKNSH.
The increase of luciferase expression actually observed (see Figure 7, line 11) in each type of cells is an additive effect: 21.0 in PC12 cells, 15.5 in NGF-PC12 cells and 23.0 in SKNSH.
Such an additive effect could not have been predicted from the prior art teachings.

Aronov *et al.* identify, in the Tau mRNA, the cis-acting region affecting stabilization of said mRNA in NGF-treated PC12 and in LAN-1 neuronal cell lines. The 241-bp fragment containing nucleotides 2519-2760 of the rat tau 3'UTR disclosed in the publication (Figure 1) has been tested in the context of the present invention as explained in the description of the patent application (page 18, lines 30-32). Aronov et al. however does not suggest combining said posttranscriptionnal regulatory element to a second distinct one, in particular to the WPRE element, in a vector according to the present invention.
It is to note that the PC12 neuronal cells used in Aronov et al. are NGF-treated cells. The authors, following observations of previous studies (Sadot et al., 1995), specifically disclose that "*the stabilization effect is enhanced by NGF treatment*" (left column, second paragraph, page 136). Aronov et al. further teach "*sequences present in fragment B may affect the stability of c-fos mRNA in transfected PC12 cells in response to NGF induction*" (see left column, last sentence of the first paragraph, page 136) and "*sequences present in fragment B and in subfragment H affect the half-life of tau mRNA, which is prolonged by NGF treatment*" (see end of right column, page 136).
The present invention surprisingly demonstrate that an highly increased expression of the transgene may be obtained in NGF-treated PC12 cells as well as in NGF-non treated PC12 cells when said transgene is operably linked, in the vector, to at least two distinct posttranscriptionnal regulatory elements, one of which is the tau 3'UTR region mentioned previously (see Figure 7 of the patent application).

Comparing more particularly the increase of expression using both Tau and WPRE, it is interesting to see that a highest increase (**16.8**) is observed in PC12 cells when compared to the increase observed in NGF-PC12 cells (5.27). Such an effect could not have been expected regarding the prior art teachings (Aronov et al. in particular).

Advantageous effects on transgene expression are clearly demonstrated in the experimental part of the application as filed wherein the claimed vectors have been concretely tested and validated for neuronal cells. These effects have further been confirmed in Brun et al. and were not predictable based on the teachings of the cited prior art.

Further, as explained in my previous declaration dated January 24, 2008, all the lentiviral vectors tested in Brun et al. (see page 788, paragraph entitled "Production of lentiviral vectors"), contain the cPPT sequence (also called the flap sequence), as do the lentiviral vectors tested by **Barry *et al.***.
The cPPT sequence increases the vector transduction efficacy by about 10 fold, due to the stimulation of the genome vector nuclear import [see Zennou *et al.*, 2001, previously submitted].
Considering that all the lentiviral vectors, including control vectors ("pTRIP-luc"), tested in Brun et al. contain the cPPT sequence, the combined effect of the cPPT sequence and of at least two, for example three, post transcriptional elements, would be a 150 to 300 folds increase of the transgene expression, relative to the expression obtained using a control vector deprived of said cPPT sequence and of post transcriptional elements.
In comparison, only a 42-fold increased GFP expression is reported in Barry et al. using a GFP virus containing both a cPPT element and a PRE element relative to their control virus which deprived of the cPPT and the PRE sequences (see the abstract). **In other words, this means that the PRE element tested in Barry *et al.* is at most responsible for a 4-fold GFP expression increase.**

The Brun *et al.* reference thus demonstrates a considerably increased efficiency of the vectors according to the present invention when compared with the vectors of Barry *et al.*.

The undersigned Declarant declares further that all statements made herein of this own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed this day of September **10**, 2009

JACQUES MALLET